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Research Article

Opportunistic and other intestinal parasites infections among HIV-positive patients in the era of combination antiretroviral therapy and preventive treatment in Ouagadougou, Burkina Faso

Abstract

Background: One of the particularities of human immunodeficiency virus (HIV) infection in the tropics is its frequent association with parasitic diseases so frequent in this area. Aim: The aim of this study was to update the epidemiological and clinical profile of intestinal parasites among HIV-positive patients in the era of combination antiretroviral therapy (ART) and preventive treatment in Ouagadougou, Burkina Faso.

Materials and Methods: A cross-sectional study was conducted in a single health care system, Yalgado Ouédraogo University hospital, between January 2013 and December 2014. A total of 131 HIV-infected patients were included in the study. Blood and Stool samples were collected for CD4 counts and for intestinal parasitic examination using direct, formol-ether concentration, a concentration method using sodium chloride solution, modified Ziehl-Neelsen and Weber modified trichrome. Odds ratio was calculated to estimate the risk attributable to different factors with confidence intervals. Chi-square (χ^2) or Fisher's Exact Test statistical analysis was used to test level of significance at $p < 0.05$ using SPSS 20.0.0.

Results: A prevalence of 73.3% of intestinal parasites was recorded. Most of the parasitic infections were protozoa (97.9%) with few helminths (2.1%). *Cryptosporidium spp.* (29.2%), was the most commonly found parasite followed by *Entamoeba coli* (23.9%) and *Isospora belli* (21.9%). There were 76 cases (78.5%) of single infection and 20 cases (21.5%) of mixed infection. Paradoxically, patients who use preventive treatment were more infected with opportunistic parasites ($p = 0.05$, OR=2.16, 95% CI= 1.06-4.40). In multivariate analysis, patients with diarrhea (OR=4.04, 95% CI=1.94-8.41), and illiterate (OR=3.70, 95% CI=1.68-8.14) had higher risk of opportunistic parasites. The diarrheic patients were 0.29 times more likely to be infected with opportunistic parasites.

Conclusion: Despite the advent of combination ART and preventive treatment of opportunistic infections, intestinal parasites remain very prevalent and associated with diarrhea among patients with HIV in Ouagadougou.

Abbreviations

AIDS: Acquired Immune Deficiency Syndrome; ART: Anti-Retroviral Therapy; CD4: Cluster of Differentiation Antigen 4; CI: Confidence Interval; CHU-YO: Yalgado Ouédraogo Teaching Hospital; HIV: Human Immunodeficiency Virus; NCPS: National Control Program on Schistosomiasis; NPELF: National Program to Eliminate Lymphatic Filariasis; OR: Odds Ratio; PCR: Polymerase Chain Reaction; p: p-value; SPSS: Statistical

Package for Social Sciences; µl: Microlitre; >: Greater than; <: Less than; %: Percent; +: positive

Introduction

One of the particularities of human immunodeficiency virus (HIV) infection in the tropics is its frequent association with parasitic diseases so frequent in this area [1-5]. This association is especially characterized by the emergence of opportunistic parasites, *Coccidia* (*Cryptosporidium parvum*,

Isospora belli), Microsporidia (*Enterocytozoon bienersi* and *Encephalitozoon intestinalis*), and *Strongyloides stercoralis*, although current parasites are also met (*Entamoeba histolytica*, *Giardia intestinalis*, *Trichuris trichiura*, *Ascaris lumbricoides*, and *Ancylostoma duodenale*) [2,5]. The resurgence of intestinal parasitic infections, previously considered being sporadic, no pathogenic or with transient pathogenic potential in immune-competent individual, continues to attract more interest in HIV-positive patients because of their high frequency and their gravity [6–9].

The spectrums of opportunistic parasitic infection vary in different regions and usually reflect the infections prevalent in these regions [10].

In Burkina Faso, in 2016, the national HIV prevalence was estimated at 0.8% (0.7–1.0) with women and youths being predominantly infected. The overall antiretroviral therapy (ART) coverage among Burkinabe people living with HIV/AIDS was estimated at 60% [11]. The first study (hospital based cross-sectional study, conducted in 1997) on intestinal parasites in patients with HIV showed that the country pays a heavy toll to the infections by these parasites (16 (19.3%) patients died, among them 11 (68.7%) had opportunistic parasites) [12]. Seventeen years later, supported measures of the intestinal parasites, knew several novelties such as: introduction of a mass treatment with Albendazole/Ivermectine in 2001, Praziquantel from 2005, respectively by the National Program to Eliminate Lymphatic Filariasis (NPELF) and the National Control Program on Schistosomiasis (NCPS); last but not least the introduction of Trimethoprim-sulfamethoxazole (SXT) preventive treatments for patients with HIV and a CD4 count <200 cells / μ L. Added to these preventive treatments, improved diagnostic techniques for parasites screening are still unknown.

The aim of this study was to update the epidemiological and clinical profile of intestinal parasites among HIV-positive patients in the era of combination ART and preventive treatment, in Ouagadougou, Burkina Faso.

Materials and Methods

Study site and study design

A cross-sectional study was conducted between January 2013 and December 2014, at the Yalgado Ouédraogo University Hospital (CHU-YO), 03 BP 7022, Ouaga 03, Ouagadougou, Burkina Faso. Socio-demographic, clinical, and laboratory data were obtained from the study subjects using a structured questionnaire and laboratory analysis of stool and blood samples.

Study population

Patients admitted in the CHU-YO were the study population. The inclusion criteria were: Patients with confirmed HIV/AIDS, with or without signs of diarrhea. The exclusion criteria were: those on specific anthelmintic, anti-protozoan, or who had treatment for intestinal parasitism in the last two weeks preceding specimen collection and those who had antacid in the last two weeks preceding specimen collection. The use of

antacids can distort protozoan morphology contributing to the difficulty in identifying the organism.

Sample collection and processing

Fresh stool samples were collected from these patients. In the first time, macroscopic analysis of stool sample was done to determine the consistency, color of the fecal material and element present in the feces such as mucus, blood, and eventually worms (*Taenia* segment). In second time, microscopic analysis was done including direct and complementary special staining methods; direct microscopy by mixing a small amount of the specimen in 0.9% sodium chloride solution was done to detect trophozoites of *Entamoeba* spp. *Trichomonas intestinalis* and *Giardia lamblia*; formol-ether concentration was used to detect cyst of protozoa (*Entamoeba* spp., *Blastocystis hominis*, *Giardia intestinalis*) and eggs of helminths (*Schistosoma* spp., *Taenia* spp.); a concentration method using sodium chloride solution (Willis method) was used to identify hookworm (*Ancylostoma duodenale*), tapeworm (*Hymenolopis nana*) and *Ascaris lumbricoides*. Weber modified trichrome was used for detection of microsporidia spores and modified Ziehl-Neelsen technique was used to detect oocysts of *Cryptosporidium* spp. and *Isospora belli*.

Intestinal parasitic infection-positive patients were defined as those who had their stool analysis positive for one or more parasites in many forms: cyst, trophozoite, eggs and/or adult.

The CD4 cells count determination was done at the laboratory of Bacteriology-Virology, largest referral center for HIV/AIDS in Burkina Faso. We categorized CD4 cell counts into 2 groups: <200 cells/ μ L and >200cells/ μ L.

Statistical analysis

The statistical analysis was done using SPSS version 20.0.0. Prevalence was calculated. Chi-square test or Fisher's Exact Test was used to determine the association between different variables in the structured questionnaire and intestinal parasitic infection. Odds ratio was calculated to estimate the risk attributable to different factors with confidence intervals. Multivariate analysis was performed to assess crude and adjusted ratio. Observed differences in data were considered significant if $p < 0.05$.

Ethical considerations

An informed consent was obtained from all study participants, after explaining the importance of the study.

Results

A total of 131 subjects were included in the study. The mean age of the study population was 40.24 years (extreme: 16 and 64 years). The sex ratio was 0.79 (Table 1).

The overall prevalence of intestinal parasites among the study population was 73.3% (96 cases). They were mostly protozoa (94: 97.9%) of which 64 cases (68.1%) were represented by opportunistic protozoa. Helminths were 2.1% (n=2). Opportunistic parasites were *Cryptosporidium* spp. with 28 cases (29.2%) followed by *Isospora belli* with 21 cases (21.9%)

and Microsporidia (*Enterocytozoon bieneusi*/*Encephalitozoon intestinalis*) with 15 cases (15.6%). Other non-opportunistic protozoa were observed in 43 patients. It was *Entamoeba coli* (n=23: 23.9%), *Giardia intestinalis* (n=9: 9.3%), *Entamoeba histolytica* (n=8: 8.3%), *Blastocystis hominis* (n=5: 5.2%) and *Trichomonas intestinalis* (n=3: 3.1%).

There were 76 cases (79.2%) of monoparasitism (single infection) and 20 cases (20.8%) of polyparasitism (mixed infection). Among the mixed infection, 15 cases (75.0%) showed the presence of coccidian (*Cryptosporidium spp.*, *Isospora belli*) or microsporidia with *Entamoeba spp.* (n=12) and *Giardia intestinalis* (n=3). There was 1 case (5.0%) of mixed infection between helminths (*Schistosoma intercalatum* + *Taenia spp.*). The remaining 4 cases (20.0%) were mixed infections of flagellates protozoa (*Trichomonas intestinalis*, *Giardia intestinalis*) and *Entamoeba coli* (Table 2).

Table 1: Demographic and clinical information's of intestinal parasites in HIV positive patients in Ouagadougou.

	Presence of parasites (%)		p-value
	No	Yes	
Ages (years)			0.8564
Mean	38.25714	40.96875	
Mean age of all participants: 40.24 ; [16-64]			
Gender			0.2324
Male (%)	19(54.3)	39(40.6)	
Female (%)	16(45.7)	57(59.4)	
Total	35(100.0)	96(100.0)	
Sex ratio: 0.79			
Place of residence			0.03886*
Urban (%)	34(97.1)	77(80.2)	
Rural (%)	1(2.9)	19(19.8)	
Total (%)	35(100.0)	96(100.0)	
Occupation			0.224
Housewife	9(25.7)	38(39.6)	
Trader	6(17.1)	11(11.4)	
Farmers	3(8.7)	7(7.3)	
Student	2(5.7)	0(0.0)	
Functionary	6(17.1)	16(16.7)	
Others	9(25.7)	24(25.0)	
Total (%)	35(100.0)	96(100.0)	
Educational Status			0.02075*
Illiterate	5(14.3)	39(40.6)	
Primary	16(45.7)	32(33.4)	
Secondary	12(34.3)	23(23.9)	
Graduate	2(5.7)	2(2.1)	
Total (%)	35(100.0)	96(100.0)	
Diarrhea			0.001167*
No	25(71.4)	36(37.5)	
Yes	10(28.6)	60(62.5)	
Total (%)	35(100.0)	96(100.0)	

* Significant association

Table 2: Prevalence of enteric parasites.

Parasites	n	%
No parasites	35	26.7
Monoparasitism (n=76)		
Protozoans (n=75)		
<i>Cryptosporidium spp. (o)</i>	21	16.0
<i>Isospora belli (o)</i>	14	10.6
<i>Microsporidia (o)</i>	13	9.9
<i>Entamoeba coli</i>	9	6.9
<i>E. histolytica</i>	6	4.6
<i>Blastocystis hominis</i>	5	3.8
<i>G. intestinalis</i>	7	5.3
Helminth (n=1)		
<i>Ascaris lumbricoides</i>	1	0.8
Polyparasitism (n=20)		
Protozoans (n=19)		
<i>Entamoeba coli</i> + <i>Cryptosporidium spp.</i>	2	1.5
<i>Entamoeba coli</i> + <i>Isospora belli</i>	6	4.6
<i>E. histolytica</i> + <i>Isospora belli</i>	2	1.5
<i>G. intestinalis</i> + <i>Cryptosporidium spp.</i>	3	2.3
<i>G. intestinalis</i> + <i>Entamoeba coli</i>	1	0.8
<i>Microsporidia</i> + <i>Entamoeba coli</i>	1	0.8
<i>Microsporidia</i> + <i>E. histolytica</i>	1	0.8
<i>T. intestinalis</i> + <i>Entamoeba coli</i>	3	2.3
Helminths (n=1)		
<i>Taenia solium</i> + <i>S. intercalatum</i>	1	0.8
Total	131	100.0

(o)= opportunistic; *E. histolytica* = *Entamoeba histolytica*; *G. intestinalis* = *Giardia intestinalis*.

There was no significant association between age and parasitic infection ($p=0.85$). But the highest prevalence of intestinal parasitic infection of 50.0% was recorded among the age group of 21-40 followed by the age group of 41-50 (33.0%). Similarly, in relation to gender and occupation, there was no significant difference ($p=0.23$ and 0.22 respectively), but the females and Housewife were more infected. Regarding the place of residence, there was a significant association between intestinal infection and the place of residence ($p=0.03$). Nineteen (19.8%) of infected patients lived in rural areas, while 77 (80.2%) in urban areas. Additionally, there was significant association with regard to educational status ($p=0.02$) (Table 1).

Statistically, a strongly significant association was found between the presence of parasites and diarrhea ($p=0.00$, OR=4.16, 95% CI=1.79-9.66) (Table 1), also between opportunistic parasites and diarrhea ($p=0.00$, OR=4.04, 95% CI=1.94-8.41). Parasitic infections were detected in 62.5% of the stool samples of HIV-positive patients with diarrhea and in 37.5% of HIV-positive patients without diarrhea.

A CD4 count lower than 200 cells/ μ L was found to be strongly associated with diarrhea ($p=0.04$, OR= 2.47, 95%

CI=1.11-5.49). Similarly, significant association was found between CD4 count lower than 200 cells/ μ L and opportunistic parasites ($p=0.02$, OR= 2.95, 95% CI= 1.28-6.81) (Table 3).

There was no significant association between opportunistic parasites and the use of preventive treatment ($p=0.05$, OR= 2.16, 95% CI=1.06-4.40), but, paradoxically, patients who use preventive treatment (SXT and albendazole) were more infected with opportunistic parasites (Table 4).

In the multivariate analysis, age, sex, occupation, residence, use of preventive treatment, profession, CD4 count less than 200 cells/ μ L, of the patients did not show significant association with prevalence of opportunistic parasites. Patients with diarrhea (OR=4.04, 95% CI=1.94-8.41), and illiterate (OR=3.70, 95% CI=1.68-8.14) had higher risk of opportunistic parasites. The diarrheic patients were 0.29 times more likely to be infected with opportunistic parasites.

Discussion

The female predominance and the younger age of our patients are consistent with the epidemiological data of HIV infection in Burkina Faso [13]. This might be due to men having less recourse to health services in Burkina Faso comparatively to women and also due to HIV epidemic mainly affecting the young and sexually active population [14].

There was a significant association between intestinal infection and the place of residence; those residing in urban areas were most infected with the prevalence of 80.2%, while the rural residence had a prevalence of 19.8%. Now, because of the tendency of people clustering around the cities, the participant's proportion from rural areas is small. On the one hand, the urban areas of Ouagadougou are the most crowded areas with a minimal level of hygiene. On the other hand, the rural areas often show more respect for sanitary conditions in the distance separating houses, animals being fenced out of town. This might explain why the lowest prevalence of

parasitism was found in rural areas and the highest prevalence in urban areas.

Like in the present study, it has been reported that a high proportion of intestinal parasitic diseases occurs in the low education level, and among housewife [12,15,16]. This could result from little information on the importance of sanitation.

Seventeen years after the first study which showed a prevalence of 61.4%, our study found that intestinal parasitism has not decreased (73.3%) [12]. However, this finding may be biased through the fact that we used screening techniques (Willis, Ritchie, chromotrope 2R) which were absent in the 1997 survey. This prevalence is also higher than that reported in Nepal (30.1%) [17]. However, our results are more in line with those found in Equatorial Guinea and in Ethiopia, where respectively 81.5% and 80.3% of the HIV-positive patients studied were infected by intestinal parasites [15,18]. Regarding epidemiological data of intestinal parasites in Burkina Faso, a high prevalence (54.7%) was also found in a review of parasitic intestinal infection reported to the Ministry of Health of Burkina Faso from 1997 to 2007. The study focused on the results of 904,733 stool examinations performed for parasite detection in public hospital parasitology laboratories [19].

The majority of parasites isolated were protozoa. The predominance of the protozoa is therefore a direct witness of the fecal peril, of the low level of individual and collective hygiene [20]. *Cryptosporidium* spp. (21.4%) was the most commonly isolated protozoa. This first place with regard to the prevalence of *Cryptosporidium* spp. was in accordance with the studies done in India [6,21,22]. The higher prevalence of *Cryptosporidium* spp., *Isospora belli* infections and other opportunistic parasites found in HIV sero-positive individuals need an intervention to avoid their opportunistic and pathogenic potentials and to provide guidelines for therapy and necessary data for planning and evaluation of care and management of HIV-positive patients. This finding also raises the question of the effectiveness of SXT preventive therapy initiated in immune-compromised patients in Burkina Faso to fight against opportunistic pathogens.

Among amoeba, the prevalence of *Entamoeba coli* (23.9%) was higher to the prevalence found in Equatorial Guinea (15.8%) [15]. The higher prevalence of *Entamoeba coli* among amoeba was also recently found in prisoners in Ouagadougou, Burkina Faso [23]. The prevalence of *Entamoeba histolytica* (8.3%) was in concordance with the finding in Nigeria and Ethiopia respectively 9.2% and 6.1%, but lower than those found in India 24.4% [5,18,22]. The prevalence rate of *Giardia intestinalis* (9.3%) was higher than an earlier study and that found in India, 4.1% [12,24]. In general, such a prevalence of these non-opportunistic parasites could have been due to water and food contamination, or with to poor personal hygiene [24]. The high prevalence of non-opportunistic parasites with HIV infected patients also draws attention to the need to include routine stool examination in HIV/AIDS management.

To our knowledge, this study for the first time showed the presence of intestinal microsporidia in Burkina Faso. But we were unable to differentiate *Enterocytozoon bieneusi* of

Table 3: Association of diarrhea and Opportunistic parasites with CD4 T-cells count<200 cells/ μ L.

		Diarrhea (%)		Opportunistic parasites (%)	
CD4 <200		No	Yes	No	Yes
	No	22(16.8)	13(9.9)	25(19.1)	10(7.6)
	Yes	39(29.8)	57(43.5)	44(33.6)	52(39.7)
		p=0.04*,OR=2.47,95% CI= 1.11-5.49		p= 0.02*,OR=2.95, 95% CI=1.28-6.81	

* Significant association.

Table 4: Relationship between the use of preventive treatment with Opportunistic parasites and diarrhea.

		Diarrhea (%)		Opportunistic parasites (%)	
Use of preventive treatment		No	Yes	No	Yes
	No	32(24.4)	23(17.6)	35(26.7)	20(15.3)
	Yes	29(22.1)	47(35.9)	34(26.0)	42(32.1)
		p= 0.04*,OR=2.25, 95% CI=1.11-4.57		p= 0.05, OR= 2.16, 95% CI=1.06-4.40	

* Significant association.

Encephalitozoon intestinalis. For this differentiation, molecular method which is not used in this survey is needed. We can nevertheless notice with many authors that these two species exist in African countries and have been estimated to vary from 1.5% to 50.0%, depending on the geographic region concerned and the diagnostic methods applied [25–27]. Since the administration of antiretroviral therapy has enabled HIV-infected patients to restore their immune status, the number of cases of microsporidia infections has decreased dramatically in developed countries [28]. This high frequency of microsporidia in our study allows us to say that, unlike some countries like the Congo where infection with those protozoa is emerging, the microsporidia are a reality in Burkina Faso [25]. But so far, they have attracted little attention from clinicians and have not been included in the SXT preventive therapy. Indeed, none of the two parasite species, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, is reported to be sensitive to this molecule [10].

Like in the present study, it has been reported the rarity of helminths in the recent surveys on intestinal parasites in Burkina Faso [23,29]. This finding was also in agreement with other studies done in African countries [5,25,30]. This low prevalence of helminths among our population is directly on the one hand, due to the treatments of mass with Albendazole/Ivermectine but also with Praziquantel led respectively by the NPELF and the NCPS, on the other hand, to extensive antiretroviral treatment administration, secondary to a stabilization of immune response. In contrast to our finding, the dominance of helminths was found in Ethiopia [24]. Whether the particular absence of *Strongyloides stercoralis*, helminth opportunistic in HIV / AIDS, can be related to its sensitivity to Albendazole, it is reported in many similar studies in the same context of treatment of mass in African countries like Nigeria, Lao People's Democratic Republic and Ethiopia [24,31–33]. This absence in our series can also be explained by the reason why we did not use a special research technique (Baermann and Lee, Koga Agar Plate, PCR) [34]. The presence of *Schistosoma intercalatum* among the few (n=2) helminths also raises a comment. Indeed, to the best of our knowledge, this study is the second which indicates the presence of this parasite species in Burkina Faso. The first detection was made in 1969 [35]. This extreme rarity is due to the fact that the West African region is not one of the usual foyers of this parasite. The usual foyers concern the rain forest areas of Central Africa [36].

In our study, diverse modalities of parasitism are observed with a monoparasitism rate (78.5%) higher than the polyparasitism (21.5%). The presence of polyparasitism was also found in other studies done in Burkina Faso, Nigeria and India where they were respectively 3.6%, 23.8% and 21.5% [5,12,37]. The presence of parasitic associations indicates one very faecal and sanitary food low level of hygiene and the unfavorable living conditions [38]. The predominance of association of protozoa is explained by the fact that these parasites often have a similar mode of infestation.

In the present study, 62.5% and 37.5% were the isolation rate for parasites in patients with and without diarrhea

respectively ($p = 0.00$). This association was in agreement with other studies which have shown that parasites are important etiological agents of diarrhea with HIV-positive patients [18,21,24].

There was a significant association between the opportunistic parasites and CD4 count <200 cells/ μ L. The presence of opportunistic parasites with the HIV-positive patients was inversely proportional to the CD4+ T-cell counts with these patients, i.e. the lower the CD4 counts the more the chances of isolation of parasites with these patients were. This finding is in concordance with other studies where decreasing opportunistic parasite positivity has been reported as CD4 count of HIV/AIDS patient increases [17,21,39,40]. This raises the question of the role of cell-mediated immunity in host defense against the parasite.

Paradoxically, in our study, patients who use preventive treatment (SXT and/or albendazole) were more infected with opportunistic parasites ($p=0.05$). However, there was no significant association between opportunistic parasites and the use of preventive treatment ($p=0.05$). The high prevalence of opportunistic parasites can be explained by the fact of inadequate immune reconstitution or poor adherence and preventive treatment interruption, among HIV+ subjects on ART.

This study gives preliminary elements to investigations for more elaborate via methods of diagnosis more successful, in particular PCR, the detection of antigen, and the iso-enzymatic analysis. In addition, a longitudinally designed study with a stronger power, incorporating additional confounding factors, would be needed to further understand the possible immunological and epidemiological mechanisms underlying HIV infection with intestinal parasite interactions.

There are some limitations of our study. This study do not precise for how long have the patients been on SXT therapy or on ART. HIV negative control groups were not included. These limitations may introduce bias. Therefore, the findings of this study, conducted in a single health care system, may not be generalizable to other health care systems and should be interpreted in light of the study limitations.

Conclusion

Despite the advent of combination antiretroviral therapy and preventive treatment of opportunistic infections, intestinal protozoa remain very prevalent and strongly associated with diarrhea among patients with HIV in Ouagadougou. Those results posit the need for considering early detection and treatment of intestinal parasites in HIV infected individuals in order to reduce their morbidity. In addition, this finding calls for establishment of specific diagnostic tests in all health laboratories and train health professionals with special attention to opportunistic intestinal parasites in HIV-positive patients.

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